

CLAIMS

(1) A method for constructing a cDNA library, comprising the steps of,

5 (a) treating the RNA sample containing mRNA and other RNA with alkaline phosphatase to remove phosphate groups from non-full-length mRNA molecules having phosphate groups at the 5'-ends,

(b) following the treatment of step (a), treating the RNA sample with acid pyrophosphatase to convert the CAP structures of the
10 full-length mRNAs in the sample into phosphate groups, wherein the full-length mRNAs have a CAP structures at their 5'-ends,

(c) following the treatment of step (b), treating the RNA sample with RNA Ligase to ligate synthetic oligo-RNA (oligo-capping linkers) to the 5'-ends of mRNAs in the RNA sample, wherein the CAP structures
15 of the mRNAs at the 5'-end are converted into phosphate groups,

(d) selecting poly (A) RNAs from the RNA sample following the treatment of step (c),

(e) performing reverse transcription using the poly (A) RNAs selected in step (d) as the templates, and the oligonucleotide
20 complementary to the synthetic RNA used in step (c) or to a portion thereof, and an oligo-d(T) adapter as the primers.

✓ (2) The method of claim 1, wherein the alkaline phosphatase used in step (a) is bacterial alkaline phosphatase (BAP).

✓ (3) The method of claim 1 or claim 2, wherein the acid
25 pyrophosphatase used in step (b) is tobacco acid pyrophosphatase (TAP).

✓ (4) The method of any one of claim 1 to claim 3, wherein the RNA sample of step (a) is total RNA.

✓ (5) The method of any one of claim 1 to claim 4, wherein the acid pyrophosphatase treatment of step (b) is performed under a
30 condition wherein the pH is higher than 6.0 and lower than 8.0.

✓ (6) A cDNA library constructed by any one of the method of claim 1 to claim 5.

✓ (7) A method for isolating a transcription regulatory region containing a promoter of a gene on the genome, wherein the method
35 comprises the steps of,

(a) determining the nucleotide sequence of a cDNA contained in

the cDNA library of claim 6,

(b) comparing the determined nucleotide sequence to a genomic DNA sequence corresponding thereto to identify the transcription initiation site on the genome,

5 (c) isolating the genomic DNA fragment located upstream of the identified transcription initiation site.